International Preliminary Examination Authority
The European Patent Office
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14 June 2006

Sent by fax

Dear Sirs

International Patent Application No. PCT/GB2005/001451
ATHERA BIOTECHNOLOGIES AB
Our Ref: ATHCZ/P32969PC

This is a response to the Written Opinion of the International Preliminary Examining Authority (IPEA) dated 18 May 2006.

Amendments

We enclose new page 19, to replace page 19 currently on file.

A typographical error has been corrected in Claim 8 ("arthothrombosis" to "atherothrombosis").

New Claim 10 specifies that the uses and the methods defined by the earlier claims are applied in respect of "*a human*" patient. It is abundantly clear that an embodiment of the present invention relates to the treatment of humans. For instance, the examples relate to the assessment of samples obtained from humans. Moreover, as explained on page 2, lines 4 to 22 is that "*A major problem associated with the therapeutic use of Annexin V ...[is its]...short half-life in the circulation of humans...In the present invention we have shown that Annexin V may stabilize atherosclerotic plaque. When Annexin V or an N-terminal fragment of Annexin V is administered according to the invention, preferably by injection, it will bind to the endothelial plaque on a first passage. The short half-life of Annexin V in the circulation is thus not a problem*". This makes it quite clear that the treatment of humans is disclosed.

New Claim 11 specifies that the uses and the methods defined by the earlier claims are applied in respect of "*a patient with vulnerable plaques*" as disclosed on page 6, lines 22 to 24.

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Novelty: Claims 1-4, and 10-11 (in part)

The IPEA have alleged that Claims 1-4 lack novelty in view of D1.

The IPEA have correctly accepted that the disclosure of D1, insofar as it relates to a *complex* of Annexin V, a radioisotope and/or an effector molecule is not novelty destroying for the present claims.

However, the IPEA have referred to paragraphs 31 and 33 as being, allegedly, relevant to the novelty of Claim 1. We respectfully disagree with the IPEA's allegation for the reasons given below.

Paragraph 31, as relied on by the IPEA, states that –

> "*The intrinsic anti-apoptotic properties of internalised annexin V could also be exploited whereby radiolabeled annexin V for imaging could be co-injected with much greater amounts of unlabeled annexin V for therapeutic effect*" (emphasis added).

What sort of "therapeutic effect"? The earlier part of the paragraph alludes to an anti-apoptotic effect. However, it is not at all clear from the contents of paragraph 31 of D1 that this is disclosure of a "therapeutic effect" *specifically in respect of atherosclerotic plaques*, as required by Claim 1 of the present application.

The only specific disclosure in D1 of the treatment of vulnerable atherosclerotic plaques is in respect of the use a *complex* of Annexin V, a radioisotope and an effector molecule to selectively kill or inactivate apoptotic cells in an atherosclerotic plaque. See D1, paragraph 32, which states its complexes can be used for "*treating vulnerable plaques*" via the activity of the effector molecule portion of the complex which "*will selectively kill or inhibit the stressed or apoptotic cells associated with the vulnerable plaque*". The IPEA have correctly accepted that the disclosure of D1, insofar as it relates to a *complex* of Annexin V, a radioisotope and/or an effector molecule is not relevant to the present claims.

Paragraph 31 of D1 merely discloses that unlabelled Annexin V can be used generally in therapy where an anti-apoptotic effect is desired. This is not a disclosure of the use of unlabelled Annexin V to prevent plaque rupture.

Moreover, it is not possible to derive from D1 that the anti-apoptotic effect provided by Annexin V could be therapeutically beneficial in the prevention of plaque rupture. On the contrary, D1 actually suggests that an anti-apoptotic effect

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in the cells of an atherosclerotic plaque would *not* be therapeutically beneficial. As discussed above, paragraph 32 of D1 teaches that its complexes can be used for "*treating vulnerable plaques*" via the activity of the effector molecule portion of the complex which "*will selectively kill or inhibit the stressed or apoptotic cells associated with the vulnerable plaque*". Selective killing or inactivation of cells in an atherosclerotic plaque is clearly the opposite of preserving such cells by preventing apoptosis. Thus, D1 teaches that, in order to treat vulnerable plaques, one should selectively kill cells therein, using the disclosed complex. The disclosure relied on by the IPEA in paragraph 31 of D1, which refers to using "*the intrinsic anti-apoptotic effects*" of Annexin V for an unspecified "*therapeutic effect*", clearly cannot be taken to be a teaching that one should use unlabelled Annexin V to prevent the rupture of atherosclerotic plaques.

Paragraph 33,as relied on by the IPEA, states that –

> "*In an alternative aspect of the present invention, the compositions may comprise or consist essentially of an annexin, such as annexin VI, coupled to otherwise bound to a targeting molecule, such as a radiolabel such as technetium-99m. The annexin is believed to both provide binding and provide a therapeutic benefit when bound to the apoptotic or stressed cells characteristic of vulnerable plaque. The annexin compositions, of course, may be further bound to porphyrin or other photodynamic or other effector molecule, generally as described above*" (emphasis added).

Two points relevant to novelty are readily apparent from the above statement –

- Paragraph 33 relates only generally to "annexins" and only specifically individualises Annexin VI, which is a different protein to Annexin V. This is clearly not a novelty destroying disclosure of any use of Annexin V, as defined by Claim 1. Moreover, paragraph 33 starts with the language "*In an alternative aspect to the present invention...*" and so it is not at all clear that one can properly combine the teachings of paragraph 33 with the disclosures that precede it.

- Paragraph 33 merely teaches a "therapeutic benefit when bound to the apoptotic or stressed cells characteristic of vulnerable plaque". What sort of therapeutic benefit is this? The reader is not told. It is quite clear that this cannot be considered to be a novelty-destroying disclosure of the

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prevention of plaque rupture, as defined by Claim 1 of the present application.

In summary, Claim 1 is quite clearly novel over the teachings of D1 as highlighted by the IPEA, and we respectfully request that the earlier objections are favourably reconsidered. Since Claims 2-4, and 10-11 (in part) depend on Claim 1, then they are clearly also novel over D1.

The IPEA have, correctly, not suggested that any other documents are relevant to the novelty of these claims.

Inventive Step: Claims 1-5, and 10-11 (in part)

The IPEA have alleged that Claims 1-4 are obvious in view of D3, and that Claim 5 is obvious in view of the combination of D3 and D7.

With respect, we disagree with the IPEA's views.

The IPEA have relied on the passage in D3 that states that "*Annexin V may be useful as a stabilizer of atherosclerotic plaques, becoming a new tool in atherosclerosis treatment*".

Of course, the question of inventive step is determined in view of what the person skilled in the art would, or would not, consider to be obvious, having regard to the disclosure of the prior art. As a result, when assessing the impact of a prior art disclosure on the issue of inventive step, one must always interpret disclosures in the prior art in the way that it is reasonable to suppose they would have been interpreted by the person skilled in the art, *without the benefit of the knowledge of the application in hand.*

So, with this in mind, we must ask the question, how would the person skilled in the art, in the absence of the knowledge of the present application, interpret D3's claim that "*Annexin V may be useful as a stabilizer of atherosclerotic plaques, becoming a new tool in atherosclerosis treatment*"? In particular, what did the person skilled in the art understand by the phrase "*stabilizer of atherosclerotic plaques*"?

The natural tendency, in light of the knowledge of the present application, is to assume that the word "stabilizer" as used in D3 is being used in the same context as the present application. However, the word "stabilize" can have more than one meaning, depending on the context in which it is used, and it is totally improper to rely on a teaching in the present application to help interpret unclear terms the

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prior art. On the contrary, the prior art must be interpreted without reliance on the teachings of the present invention.

In D3, the word "stabilize" is used in relation to the report that Annexin V can reduce apoptotic activity in atherosclerotic plaque. Apoptosis is a form of cell death and reduction in apoptosis will result in reduced cell death and a "stabilisation" of the cell population of the plaque. This is how the person skilled in the art would understand the disclosure of D3 – i.e., they would consider that D3 teaches the use of Annexin V to stabilise the cell population by prevention of apoptosis.

The stabilisation of a cell population in an atherosclerotic plaque by reduced apoptosis *cannot be equated with the prevention of plaque rupture*. There is absolutely no evidence in the art, or provided by the IPEA, that the prevention of apoptosis in an atherosclerotic plaque will lead to the prevention of plaque rupture. One simply cannot equate the two processes.

As already explained above, the teaching of D1 suggests that the "*intrinsic anti-apoptotic effects*" of Annexin V, if used in the unlabelled form in an atherosclerotic patient, would have the opposite effect to the *complex* of Annexin V, a radioisotope and an effector molecule as disclosed in D1. The complex is disclosed to treat vulnerable plaques by selectively killing or inactivating apoptotic cells in an atherosclerotic plaque promote plaque rupture. By contrast, both D3 and D1 disclose that unlabelled Annexin V has an anti-apoptotic effect, i.e. according to D3 and D1's disclosure it would *preserve* the apoptotic cells in an atherosclerotic plaque. D1's teaching is that such preservation is not desirable in treating vulnerable atherosclerotic plaques.

Additionally, we enclose Merched *et al*, 2003, *Arterioscler. Thromb. Vasc. Biol.*, 23, 1608-1614. This document reports that –

- macrophages are the major cellular components of atherosclerotic plaques and they undergo both proliferation and apoptosis, processes tightly regulated by the tumour suppressor protein p53 (see page 1608, left-hand column, first paragraph);

- macrophage proliferation in atherosclerotic lesions (plaques) occurs more readily in the absence of p53 expression (page 1611, left-hand column, line 6 *et seq*); and

- p53 expression confers stability to plaques, whereas the absence of p53 expression renders plaques more vulnerable to rupture (page 1612,

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paragraph bridging left and right columns, particularly the final two sentences).

In short, Merched *et al* show that an absence of p53 expression correlates both with high macrophage proliferation and with increased risk of plaque rupture. In other words, according to the teaching of Merched *et al*, increased macrophage proliferation appears to be related to an increased risk of plaque rupture. Increased macrophage proliferation would be expected to be the result of the *prevention* of macrophage *apoptosis*. Annexin V is thought to provide an anti-apoptotic effect generally (see D1) including in the cells of plaques (see D3). Therefore, it follows that, in light of Merched *et al*, the expected effect of Annexin V treatment on plaques would be an *increased* risk of rupture. This is directly opposite to the present invention.

In view of this, the person skilled in the art would not interpret the disclosure in D3 that "*Annexin V may be useful as a stabilizer of atherosclerotic plaques*" (emphasis added) as an indication that plaque rupture could be prevented by Annexin V. On the contrary, it is clear that the person skilled in the art would consider this statement to be a teaching that Annexin V could be used to stabilise the cell population of a plaque by prevention of apoptosis.

The IPEA are, with respect, reminded that the language used in Claim 1 specifies that the defined composition is used to prevent plaque rupture. The claim does not use the term "stabilize" and so there can be no lack of clarity in respect of the claim.

The fact that the description of the present application, and the prior art, use the same word "stabilise", does not mean that they disclose the same information. On the contrary, the present application and D3 use the term "stabilise" in different contexts to refer to different biological processes.

Based on the teaching of D3, there is absolutely no suggestion, and more importantly, the person skilled in the art would have no reason to suspect, that Annexin V compositions could be used to prevent the rupture of atherosclerotic plaques. Rather, D3 merely teaches the person skilled in the art that Annexin V can be used to stabilise cell numbers in plaques by reducing apoptosis. The person skilled in the art has no reason to think that this would result in the prevention of plaque rupture.

Accordingly, Claim 1 is inventive over D3.

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Likewise, Claim 1 is inventive over D1 for the same reasons, i.e. there is no suggestion in D1 that Annexin V compositions could be used to prevent the rupture of atherosclerotic plaques. In fact, the teaching of D1 suggest the opposite.

Claims 2-5, and 10-11 (in part) depend on Claim 1 and so are inventive over both D1 and D3 for the same reasons as Claim 1.

Novelty and Inventive Step: Claims 6 to 9, and 10-11 (in part)

The IPEA have alleged that Claim 7 lacks novelty in view of D5 and D6.

With respect, we fail to understand the IPEA's position in respect of D5. As pointed out in our previous response to the Written Opinion of the ISA, paragraph [0023] of D5 suggests that the coronary syndromes that are to be treated include "*unstable and angina pectoris, non-Q wave myocardial infarction and Q-wave myocardial infarction*". It is then stated that "*These syndromes are believed to be the common pathophysiological substrate caused by a rupture of an atherosclerotic plaque in one of the coronary arteries*" (emphasis added).

Hence it is clear that the relevant disclosure in D5 does not relate to the prevention of plaque rupture *as such*, but rather just conditions that may *result* from plaque rupture, i.e. conditions that may follow *after plaque rupture has occurred.*

Likewise, the IPEA's own interpretation of D5 is that it relates to the use of IvIg for the treatment of conditions *caused by the rupture of an atherosclerotic plaque*.

Since the conditions treated by D5's teaching are *caused by* rupture of atherosclerotic plaques then it logically follows that atherosclerotic plaque rupture *has already occurred.* We fail to understand how this teaching can possibly be construed as the disclosure of a method of preventing plaque rupture! Clearly it cannot. In fact, the methods of D5 presuppose that plaque rupture has already occurred, and thereby those disclosures necessarily relate to methods that have failed to prevent plaque rupture. Thus, it will be clear to the IPEA that D5 relates to the treatment of *different conditions* to those defined in Claim 7. Accordingly, Claim 7 must be novel over D5.

As regards D6, we had previously pointed out in our response to the Written Opinion of the ISA that D6 appears to relate to the use of IvIg in the prevention of atherosclerosis *as such*, and thus discloses the *reduction of the growth atherosclerotic plaques*, which is quite distinct from the subject-matter of Claim 7, which relates to the prevention of rupture of atherosclerotic plaques that have already formed.

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As we understand the IPEA's position on this matter, a prior art teaching that allows the prevention of the growth of atherosclerotic plaques is considered to be indistinguishable with a method of preventing the rupture of plaques.

With respect, we disagree. One cannot prevent the rupture of a plaque if it does not exist. The teaching of D6 is, in effect, a method of reducing the formation of plaques in the first place. By contrast, the present invention relates to methods for preventing the rupture of plaques that have *already formed*. These are two wholly distinct biological processes and there is absolutely nothing in D6 that teaches or even suggests that IvIg can be used to prevent the rupture of already formed atherosclerotic plaques.

Thus, it is clear that Claim 7 is novel over D6.

Moreover, neither D5 nor D6 suggest that it would be possible to prevent the rupture of pre-existing plaques by the use of IvIg. The teachings of D5 and D6 are wholly irrelevant to the subject-matter of Claim 7, and so it follows that Claim 7 is inventive over D5 and D6, either individually or in combination.

Claim 9, insofar as it is dependent on Claim 7, is clearly inventive over D5 and D6 for the same reasons.

The IPEA have also alleged that Claims 6 and 8 lack novelty over the disclosures of D9 to D11. The basis of the IPEA's allegation is that D9 to D11 allegedly disclose the use of the antibody EO6/T15 to achieve "an atheroprotective effect".

However, as discussed above in respect of D6, one *cannot* conclude that the teaching of the use of antibodies to achieve "an atheroprotective effect" is a disclosure of the use antibodies to *prevent the rupture of atherosclerotic plaques*. On the contrary, the use of antibodies to achieve an atheroprotective effect (and thereby reduce plaque *formation*) is clearly not the same as the prevention of rupture of a plaque that has already been formed.

Moreover, D9 to D11 further differ from the claimed invention in that they relate exclusively to investigations in mice into the effect of *vaccines* comprising *S. pneumoniae* or components derived therefrom, on the development of atherosclerosis.

The documents report that one of the effects of vaccination is an increase in the production of the antibody EO6/T15. However, the authors of D9 do not show that the atheroprotective effect that is observed in response to immunisation with

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either *S. pneumoniae* or MDA-LDL can necessarily be specifically attributed to the increased production of the EO6 antibody. EO6 is just one of a very large number of antibody types that show increased production in response to the use of these vaccines. The use of complex vaccines such as *S. pneumoniae* and MDA-LDL will inevitably result in complex immune responses, which will even include non-antibody related responses. The person skilled in the art would appreciate that any number of different antibodies, or other immune responses, raised by administration of these vaccines could be responsible for the atheroprotective effect reported in D9 and that successful reproduction of the observed effect might be altered if a different immune response were to be induced. D10 and D11 provide the same teaching as D9.

D9 to D11 only teach that the observed effect on atherosclerosis can be achieved using the route of vaccination to provoke an immune response in mice. There is absolutely no teaching or suggestion in D9 to D11 that an equivalent effect could be obtained by the administration of particular antibody preparations.

Bearing in mind that the skilled person is "*considered to be conservative*" and would not *"try to enter unpredictable areas nor take incalculable risks*" (see the EPO's "White Book", i.e. The Case Law of the Boards of Appeal of the EPO, 4th Edition, section I.D.5.1.3, page 111 of the English language version), it is clear that, to the extent that D9 to D11 motivate the person skilled in the art to attempt to treat atherosclerosis at all, then it is motivation to do so using either *S. pneumoniae* or MDA-LDL as vaccines, in order to ensure that the same complex immune responses as reported in D9 to D11 are provoked in his subject. The skilled person has no reason to deviate from this teaching – there is certainly no motivation in any of D9 to D11 for him to do so. Indeed, there is a considerable disincentive for the person skilled in the art to deviate from the exact teaching of D9 to D11, because he would understand that deviation from the identity of the vaccine used could lead to a completely different immune response. The ability of different vaccines, or in the present case a non-vaccine route that involves the direct administration of an antibody preparation, to provide an atheroprotective effect would be an "*unpredictable area*" with "*incalculable risks*" of failure. The cautious and conservative skilled person *would*, therefore, continue to use either *S. pneumoniae* or MDA-LDL as vaccines.

In light of the above comments, the IPEA will appreciate that D9 to D11 are totally irrelevant to a method that involves the administration of antibody sub-fractions for any reason (since D9 to D11 motivate the person skilled in the art to use specific *vaccines*), much less for the purpose of preventing the rupture of pre-formed atherosclerotic plaques.

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Accordingly, it will be readily apparent to the IPEA that Claims 6 and 8 are novel and inventive over the teachings of D9 to D11, alone or in combination.

Claims 10-11 depend (in part) on Claims 6-9 and so are novel and inventive over the cited art for the same reasons as discussed above.

Moreover, Claim 10 is limited to the treatment of humans. The prior art disclosures of D9-D11 are directed to observations made *in mice*. The cited prior art makes it absolutely clear that the views of those skilled in the art are that it is *extremely uncertain* as to whether the findings in mice can be applied to humans.

D10 explicitly suggests that the authors are uncertain as to whether its teachings could be successfully transferred from mice to humans. See page 741, the final paragraph of the "Discussion" section –

> "*Much less is known about the human response ... Although the results outlined in this report suggest a protective effect, this could primarily derive from the fact that the dominant antibody response in mice is IgM. The human immune response is more complex, and available pneumococcal vaccines have not been developed to optimise the IgM response to cell wall polysaccharide*[40]. *Moreover, although the development of IgM responses may be beneficial, T-cell-dependent IgG responses to phosphorylcholine may lead to other, possibly adverse, effects (such as increased foam-cell formation after uptake of IgG-oxLDL immune complexes by Fc-γ receptors)*".

Thus, not only does D10 suggest that the short-term atheroprotective observations from mice may not be applicable to humans, but D10 also further suggests that a similar type of treatment in humans may even be atherogenic. This is a clear disincentive to the person skilled in the art to attempt to treat atherosclerosis in humans by increasing the levels of oxLDL antibodies.

Likewise, D11 also casts doubt on whether the short-term atheroprotective method developed in D10 for mice could be successfully transferred to humans. See D11, page 642, second column, lines 16-19 –

> "*Before seriously considering whether we can prevent atherosclerosis with a pneumococcal vaccine, the function of antibodies to oxLDL must be defined in humans*" (emphasis added).

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The above-quoted passage of D11 makes it absolutely clear that the person skilled in the art would not "*seriously consider*" attempting to treat or prevent atherosclerosis in humans by the method of D10. This is further evidence that a person skilled in the art, upon reading D9-D11, would have had *no reasonable expectation* of successfully treating atherosclerosis in humans using this technique. Nor is there any indication in these documents that one could, much less should, modify the *vaccination-based* method of D9-D11, in order to arrive at a method or use as defined by Claim 20, specifically for the prevention of atherothrombosis and/or plaque rupture in a human

Thus, it will be clear to the IPEA that Claim 10 is novel and inventive.

Likewise, Claim 11 is limited to the treatment of a patient with vulnerable plaques. All of D6 and D9-D11 refer to the treatment or prevention of atherosclerosis, i.e. reduction of plaque formation. As discussed above, this is quite distinct from the prevention of plaque rupture as defined by the present claims. Claim 14 is specifically limited to the treatment of patients "with vulnerable plaques". Accordingly, it is clear that Claim 14 relates to the treatment of a physiologically distinct group of patients in order to achieve a distinct clinical outcome (i.e. prevention of atherothrombosis and/or plaque rupture). Thus, it will be clear to the IPEA that Claim 11 is novel and inventive.

The IPEA have also alleged that the application has failed to provide proof that the technical problem posed, which according to the IPEA can be defined as the "*provision of an agent to prevent atherothrombosis and/or plaque rupture*", has actually been solved by any purified subfraction of pooled immunoglobulin.

However, the IPEA is incorrect. The data in the application provides clear evidence that a subfraction of pooled immunoglobulin, IvIg, can be used to enhance Annexin V binding in patients that possess endogenous antibodies that would otherwise disrupt Annexin V binding.

We refer the IPEA to the results on pages 13-14 of the application, which report that –

(a) IgG samples that have been depleted (i.e. aPC antibodies removed) allow more Annexin V binding than the same antibody sample in which aPC antibodies are present, i.e. aPC can inhibit Annexin V binding (page 13, lines 15-20).

(b) Plasma from SLE patients can inhibit Annexin V binding, but this can be avoided by removing aPC antibodies from the plasma, i.e. aPC is responsible for the inhibition observed when SLE-derived plasma is used (page 13, lines 22-28).

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(c) Annexin V binding to human umbilical venous endothelial cells (HUVECs) can be inhibited by the presence of such serum, but that this inhibitory effect can be avoided by preincubation with the immunoglobulin preparation IvIg, which shows that antibodies in an IvIg preparation can neutralise those antibodies in patient's serum that block the binding of Annexin V (page 14, lines 7-12).

Thus, the data in the application readily support the use of a subfraction of pooled immunoglobulin to promote Annexin V binding, by counteracting the inhibitory effect of a patient's endogenous antibodies.

As explained at page 15, lines 9-19, Annexin V is present in atherosclerotic lesions at many sites, especially those that are prone to plaque rupture, and when Annexin V binding is not optimal (e.g. due to inhibition by endogenous antibodies) then the risk of plaque rupture is strongly raised. The data in the present application provides a clear indication that subfractions of pooled immunoglobulin can be used to overcome the inhibitory effect of certain endogenous antibodies and promote Annexin V binding, thereby reducing the risk of plaque rupture.

Other Matters

In relation to the IPEA's comments regarding industrial applicability and under Item VIII of the Written Opinion, we believe that these do not require further comment at this stage and will be better dealt with during national prosecution. Any amendment is not to be construed as abandonment of subject matter.

We look forward to the receipt of a favourable International Preliminary Report on Patentability (Chapter II). However, should the examiner not be inclined to agree with these observations, we should appreciate the opportunity to make further representations, either by way of a telephone discussion or a further written

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response prior to the issuance of the relevant report.

Yours faithfully

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For and on behalf of Eric Potter Clarkson LLP

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